# THE LANCET
# Infectious Diseases

## Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

# Appendix

**Supplementary table 1. HIGH RISK GROUP QUESTIONNAIRE: ABATTOIR WORKER**

## GENERAL INFORMATION

1. **Subject ID:**___ ___ ___ ___ ___ ___ ___ ___

2. **Interviewee Name:** First name _____ Surname_____

3. **Date of interview** (*dd/mm/yyyy*): ____/____/____

4. **Location** (*Region, City, Province*): _____

5. **Language used for interview:** ☐ English ☐Hausa ☐ Fulani ☐ Other _____

6. **Translator needed for interview?** ☐ **YES** ☐ **NO**

7. **Gender (tick one):**☐ Male ☐ Female

8. **Area of primary residence of subject (address):** _____

9. **Age of interviewee (in years):** _____

10. **What is your current marital status?** ☐ Single ☐Married ☐ Divorced ☐Widowed

11. **How many people live in your household with you (one household is defined as sharing a single kitchen)?**

    i. Children aged less than 18 years old: _____

    ii. Adults aged 18 years and older: _____

## OCCUPATIONAL EXPOSURES

12. **How long have you worked at this abattoir?** ___ ___ Years ___ ___ Months (**IF NOT WORKING IN ABATTOIR GO TO QUESTION 19**)

13. **What is/are your job/jobs at this abattoir? (tick all that apply)** ☐ Slaughtering of animals ☐ Cleaning ☐ Product (meat) storage/etc ☐ Other _____

    Of the listed options, which you selected, which is your primary job?

    _____

14. **How many days of the week do you work at this abattoir?**_____

15. **Do you hold other jobs aside from working at the slaughterhouse?**☐ **YES** ☐ **NO**

    If yes, what is/are your other job(s)? _____

16. **If your job requires dealing with animals in the abattoir, indicate which animals you regularly have contact with as part of your work:**

| Animal species | Contact with excrement (ie. feces or urine) | Dead, alive, both (circle one) |
|---|---|---|
| A Camels | Yes No | Dead Alive Both |
| B Sheep | Yes No | Dead Alive Both |
| C Goats | Yes No | Dead Alive Both |
| D Cattle | Yes No | Dead Alive Both |
| E Horses | Yes No | Dead Alive Both |

| | | | |
|---|---|---|---|
| F Donkey | Yes   No | Dead   Alive   Both | |
| G Other_____ | Yes   No | Dead   Alive   Both | |

## PERSONAL PROTECTIVE EQUIPMENT AND HYGIENE PRACTICES WHEN WORKING IN ABATTOIR

17. **What personal protective equipment do you usually wear when working at the abattoir?**
    ☐ No protective equipment used    ☐ Gloves    ☐ Coveralls    ☐ Dust masks
    ☐ Boots or boot covers    ☐ Respirators    ☐ Eye protection (goggles, safety glasses)
    ☐ Other:_____

18. **How often do you usually wash your hands while working at the slaughterhouse (check all)**

    ☐ At meal times    ☐ Before and after each animal related task    ☐ At bathroom times

    ☐ The beginning and end of the day    ☐ Rarely

## ANIMAL EXPOSURES IN/AROUND THE HOME  (where you live)

19. **Were any livestock (e.g. camels, sheep, goats, cattle, horses) kept in or around your home in the last six months?** ☐ YES    ☐ NO    ☐ UNKNOWN

    *Name the species, the number of animals and what they are used for?*

| Animal species | Number of animals | What they are used for? (circle one) | Did you have direct contact (i.e., touch) with these animals? |
|---|---|---|---|
| Camels | None<br>< 10 animals<br>≥ 10 animals | Food    work<br>racing    pets | Yes   no   unknown |
| Sheep | None<br>< 10 animals<br>≥ 10 animals | | Yes   no   unknown |
| Goats | None<br>< 10 animals<br>≥ 10 animals | | Yes   no   unknown |
| Cattle | None<br>< 10 animals<br>≥ 10 animals | | Yes   no   unknown |
| Horses | None<br>< 10 animals<br>≥ 10 animals | | Yes   no   unknown |

20. **In the last six months, did you have any contact with any carcasses, body fluids, secretions, urine or excrement of camels in or around your home?**    ☐ YES    ☐ NO    ☐ UNKNOWN

21. **In the last six months, did you have any contact with any camel bedding, stray or feed in or around your home?**
    ☐ YES    ☐ NO    ☐ UNKNOWN

22. **At your home did you do any of the following activities , in the last six months:**

    | | | | |
    |---|---|---|---|
    | a. Feed camels? | ☐ YES | ☐ NO | ☐ UNKNOWN |
    | b. Clean camel housing? | ☐ YES | ☐ NO | ☐ UNKNOWN |
    | c. Clean farm equipment? | ☐ YES | ☐ NO | ☐ UNKNOWN |
    | d. Slaughter camels? | ☐ YES | ☐ NO | ☐ UNKNOWN |
    | e. Assist with the birth of camels? | ☐ YES | ☐ NO | ☐ UNKNOWN |
    | f. Milk camels? | ☐ YES | ☐ NO | ☐ UNKNOWN |
    | g. Other tasks? | ☐ YES | ☐ NO | ☐ UNKNOWN |

    If yes, please specify:_____

23. **Do others living in your household (e.g., domestic help or relative) frequently visit or work on a farm or market where camels are kept or sold?** ☐ YES ☐ NO ☐ UNKNOWN

## FOOD EXPOSURES

*The following series of questions are focused on food exposures in the last six months*

24. **Do you regularly drink unpasteurized camel milk?** ☐ YES ☐ NO ☐ UNKNOWN
25. **Do you use camel urine for medicinal purposes?** ☐ YES ☐ NO ☐ UNKNOWN
26. **Do you drink camel urine?** ☐ YES ☐ NO ☐ UNKNOWN

## TRAVEL HISTORY AND EXPOSURES

27. **During the last six months have you travelled outside Kano metropolis?** ☐ YES ☐ NO ☐ UNKNOWN

*If yes, what countries/regions have you visited?*

| *Country* | *Region/City* | *Approximate Dates* |
|---|---|---|
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |

28. **Have you attended any recent mass gatherings (e.g., weddings, festivals or religious pilgrimages) outside of your regions country where there were large numbers of people together?** ☐ YES ☐ NO ☐ UNKNOWN

*If yes, specify event(s) and location:*

_____

## PAST MEDICAL HISTORY

29. **Have you been hospitalized with respiratory illness in the past 1 year?**
☐ YES ☐ NO ☐ UNKNOWN
30. **If Yes to above, where were you hospitalized during the course of your illness?**
☐ YES ☐ NO ☐ UNKNOWN

If yes, when were you hospitalized (MM/YYYY):      ___/_____

If yes, which hospital did you receive treatment(s)? (name and address)
_____

**May we contact you again with follow up questions or clarifications?** ☐ **YES** ☐ **NO**

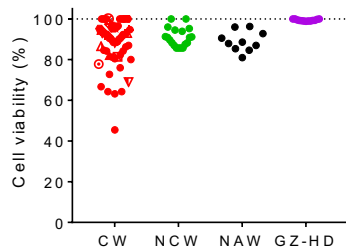31. **If yes, telephone number of subject:** _____

Figure S1

A  B

**Fig S1. Abattoir in Kano, Nigeria.** (**A**) A landscape map of the slaughterhouse was based on a google map showing the locations of the slaughterhouses for camels and for other animals such as sheep or cattle. (**B**) Workers slaughtering camels illustrating the close exposure the CWs to the camel carcasses and the lack of personal protective equipment.

# Figure S2
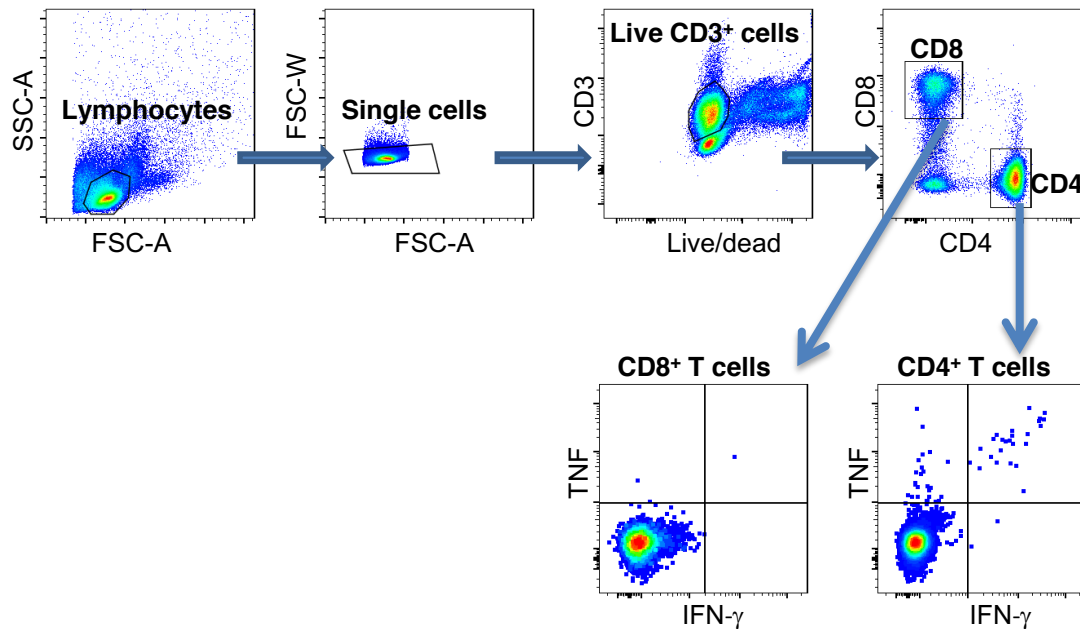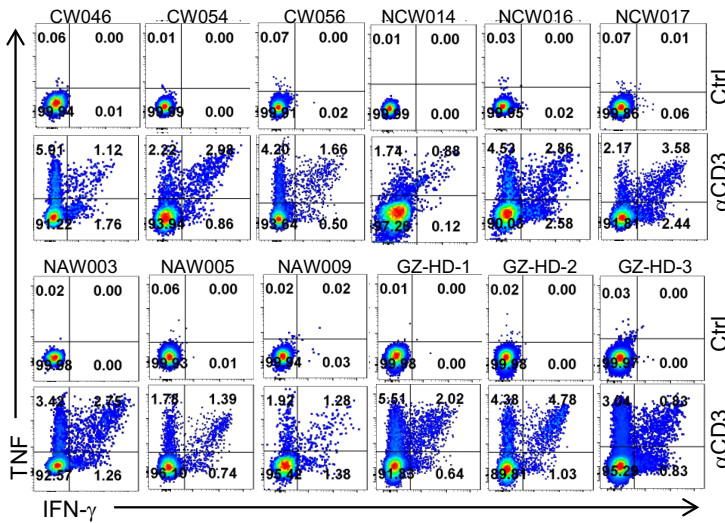
**A**   Cell viability



**B**   T cell assay gating strategy



**Figure S2. Cell viability and gating strategy for MERS-CoV specific T cells in human PBMCs.** PBMCs were counted with trypan blue staining and then stimulated with MERS-CoV peptide pools for 12 h in the presence of brefeldin A. Cells were then labeled for cell surface markers, fixed/permeabilized with Cytofix/Cytoperm Solution and labeled with anti-intracellular cytokine/protein antibodies. All flow cytometry data were acquired on a BD FACSVerse and analyzed using FlowJo software. Cell viability (**A**) and gating strategy (**B**) are shown.
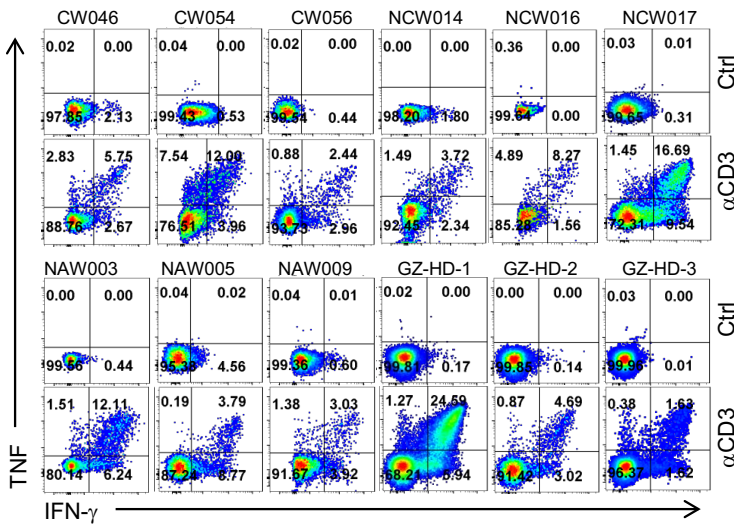
# Figure S3



**Fig S3. PBMCs from Nigeria CWs, NCWs, NAWs and GZ-HDs were activated by anti-human CD3 antibody (aCD3).** PBMCs from CWs, NCWs, NAWs and GZ-HDs were stimulated with anti-human CD3 antibody (10 $\mu$g/ml) for 12 h in the presence of brefeldin A to serve as positive control. Frequencies of activated CD4$^+$ (**A**) and CD8$^+$ (**B**) T cells (determined by IFN-$\gamma$ and TNF intracellular staining) from CWs (CW046, CW054, and CW056), control NCWs (NCW014, NCW016, and NCW017), NAWs (NAW003, NAW005, and NAW009) and GZ-HDs (GZ-HD1, GZ-HD2 and GZ-HD3) are shown.
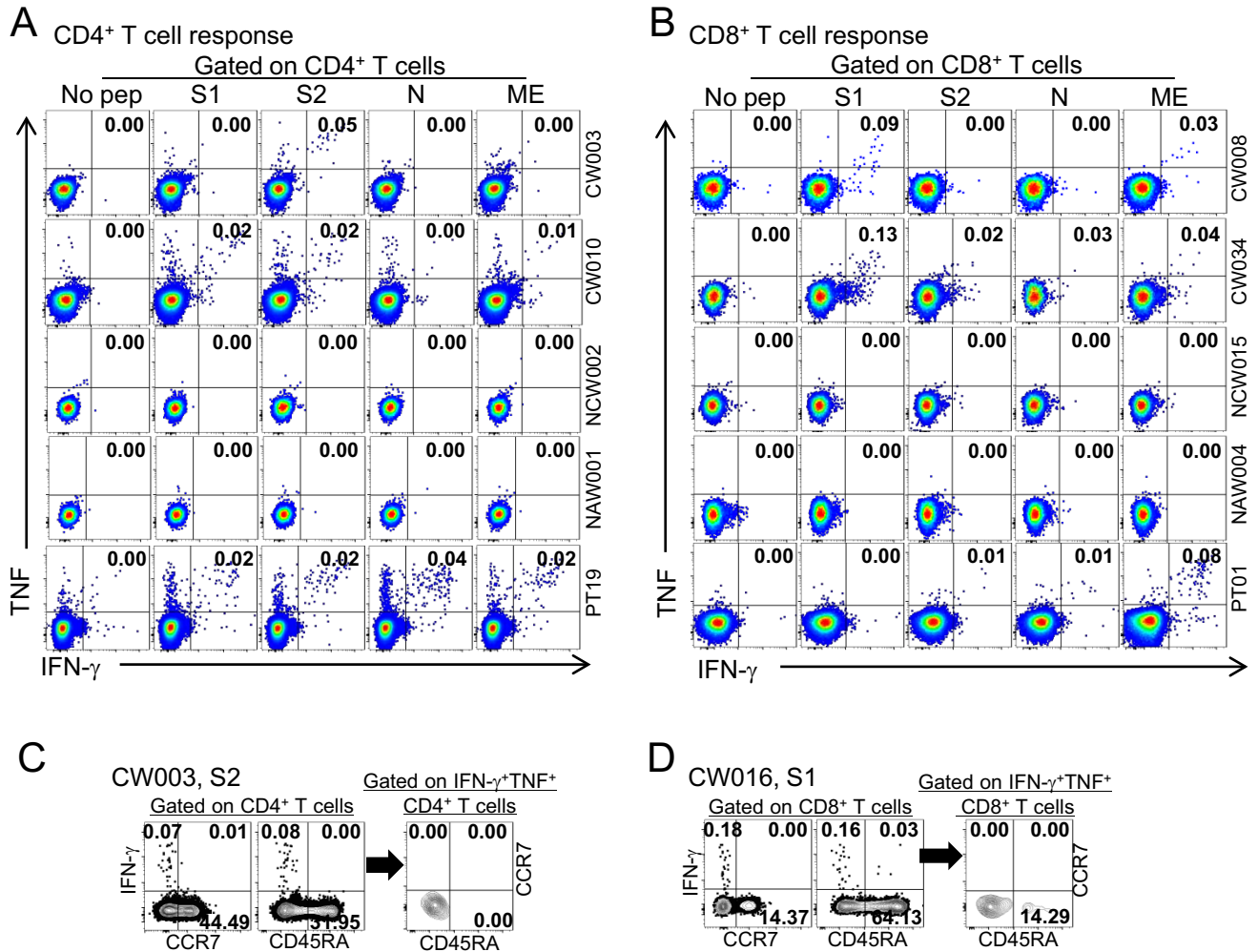
# Figure S4



**Fig S4. Gating strategies for the MERS-CoV-specific CD4+ and CD8+ T cell responses in camel workers and controls.** PBMCs from camel abattoir workers (CW), non-camel abattoir workers (NCW) and non-abattoir workers (NAW) were stimulated with MERS-CoV structural protein-specific peptide pools for 12 h in the presence of brefeldin A. T cell responses were assessed as cells expressing both interferon-γ (IFN-γ) and tumor necrosis factor (TNF). PBMCs collected 6-24 months after virologically confirmed MERS-CoV infections in Saudi Arabia were included as positive controls (PT). Examples for gating of MERS-CoV-specific CD4+ (A) and CD8+ (B) T cells as well as the effector memory (CD45RA⁻CCR7⁻) cells of virus-specific CD4+ (C) and CD8+ (D) T cells are shown.
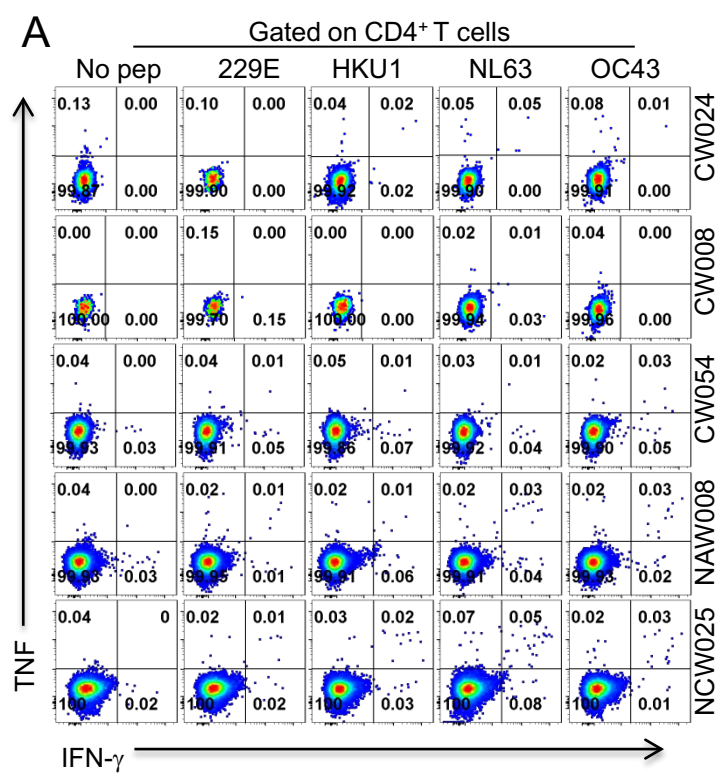
# Figure S5



**Fig S5. Gating strategy for the specific T cell responses against different human coronaviruses in camel workers and controls.** PBMCs of CWs, NCWs, NAWs from Nigeria were stimulated with structural peptide pools from human CoVs 229E, HKU1, NL63, and OC43. Examples for gating of specific CD4+ T cells are shown.